High-throughput analysis of morpho-physiological traits contributing to drought stress response in *Arabidopsis thaliana* using PlantScreen^{TM} Compact System

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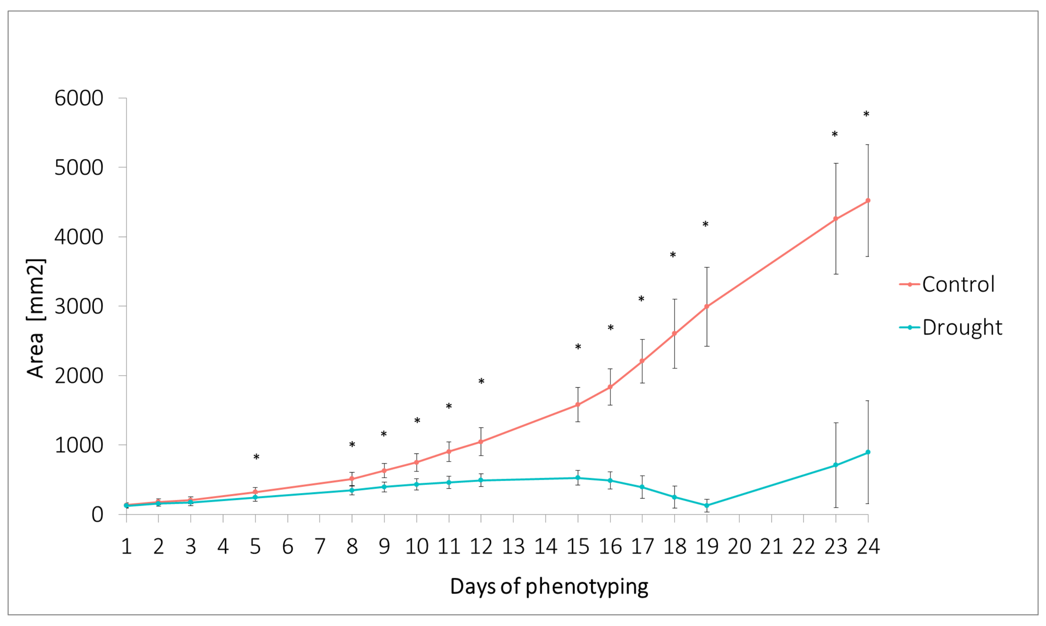
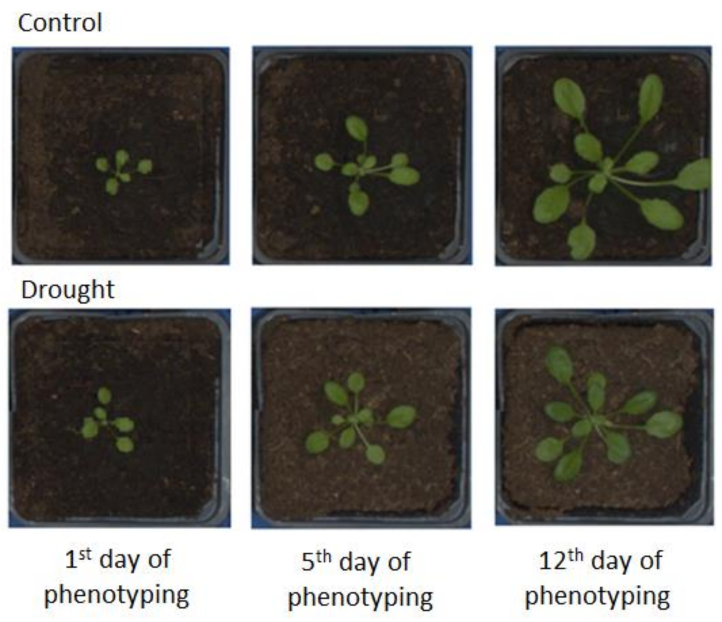
Overview

Drought is one of the main stress factors severely affecting the agriculture land in global scale and causing significant reduction of plant growth and yield. To enhance our understanding of the early responses to drought, we designed an experimental protocol based on automated integrative analysis of photosynthetic performance, growth analysis and morphological traits analysis at the onset and early phase of drought stress response in *Arabidopsis thaliana* ecotype Columbia grown in soil. Here we present optimized experimental procedure for dynamic quantitative analysis of structural and physiological phenotypes very early upon stress imposition. Results for control and drought stressed group are shown. In general, drought significantly and rapidly affected photosynthetic performance and impacted growth dynamics of *Arabidopsis* plants at different stages of stress response.

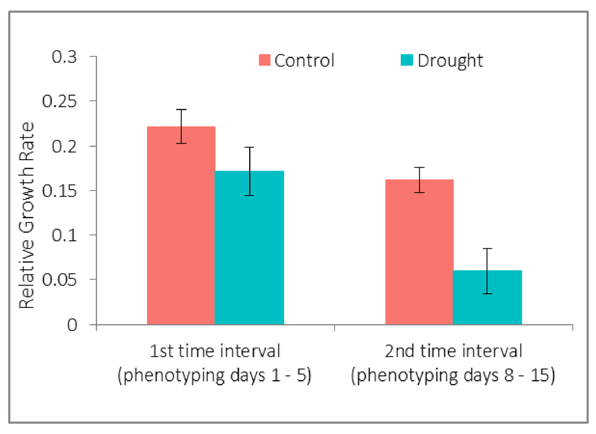
Results

RGB Imaging



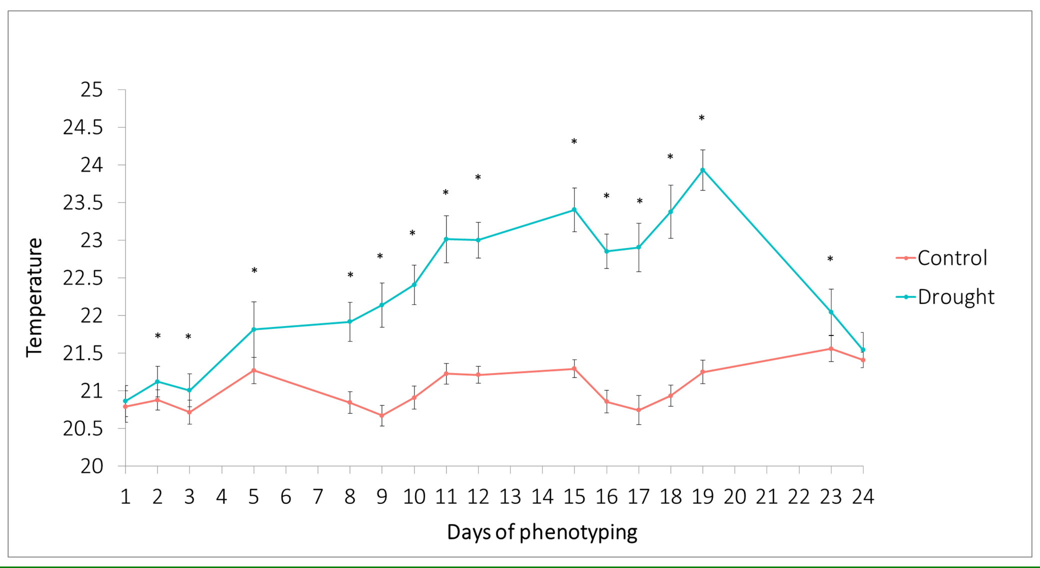


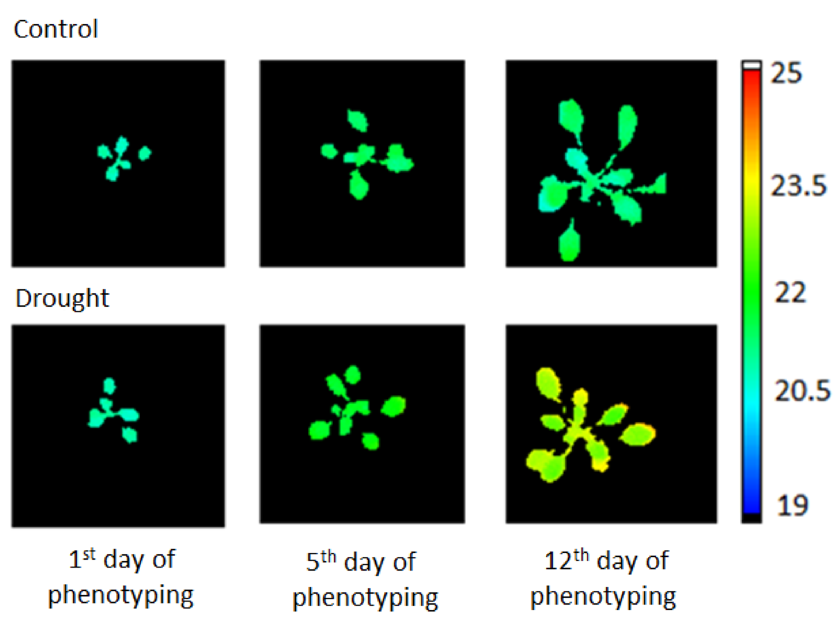
Growth rate in drought treated plants was significantly reduced 7 days after last watering (phenotyping day 5). Following day 12 of phenotyping, stressed plants stopped growing and started to wilt as shown by reduced size, that was in 60% of plants recovered upon re-watering.



Relative growth rate was calculated for the early phase of growth and stress response (DAS 17 to DAS 21 , 1-5 days of phenotyping) and later phase (DAS 22 to DAS 31) showing slower growth and strong reduction in growth rate in drought treated plants in the second phase of experiment.

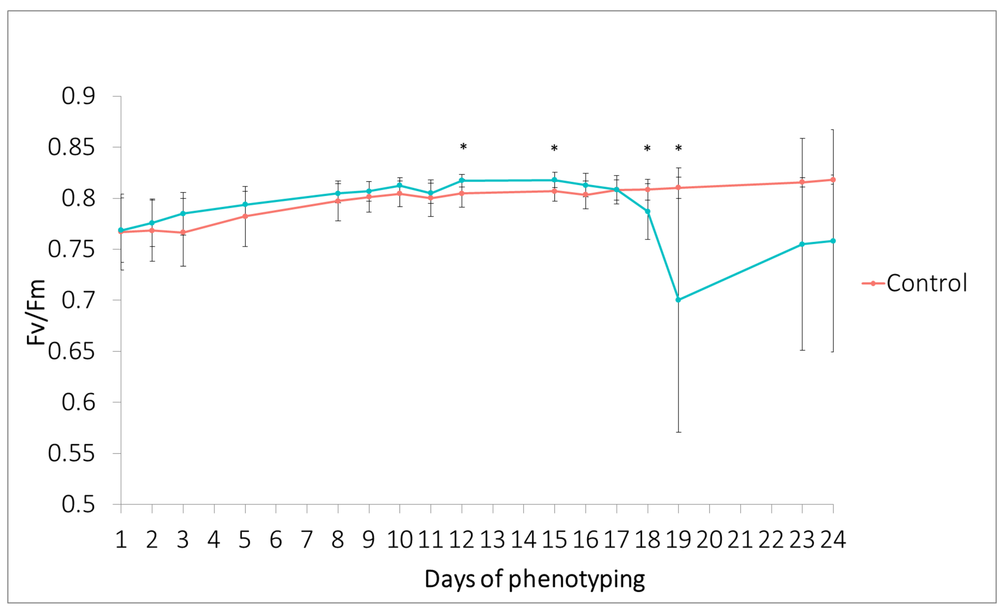
Thermal Imaging

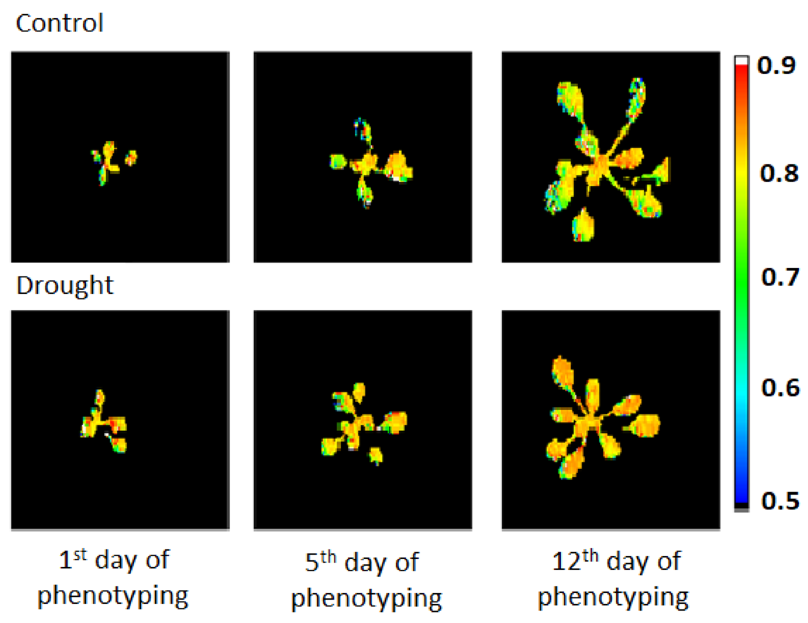




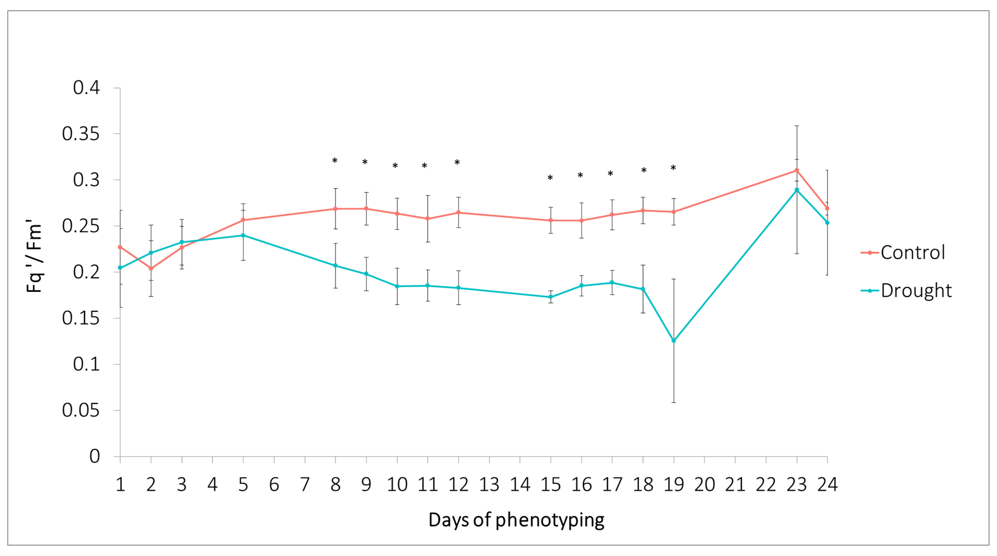
Significant difference in average temperature of the plants was evident from second day of phenotyping, which was 4 days from last watering of stressed plants. The temperature of surviving plants after the re-watering step (phenotyping day 19) rapidly decreased to control levels temperature.

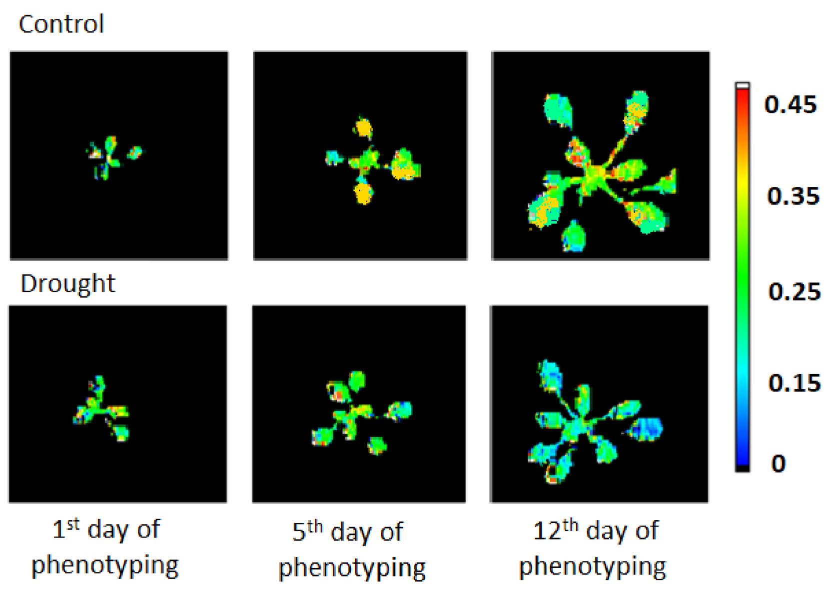
Fluorescence Imaging



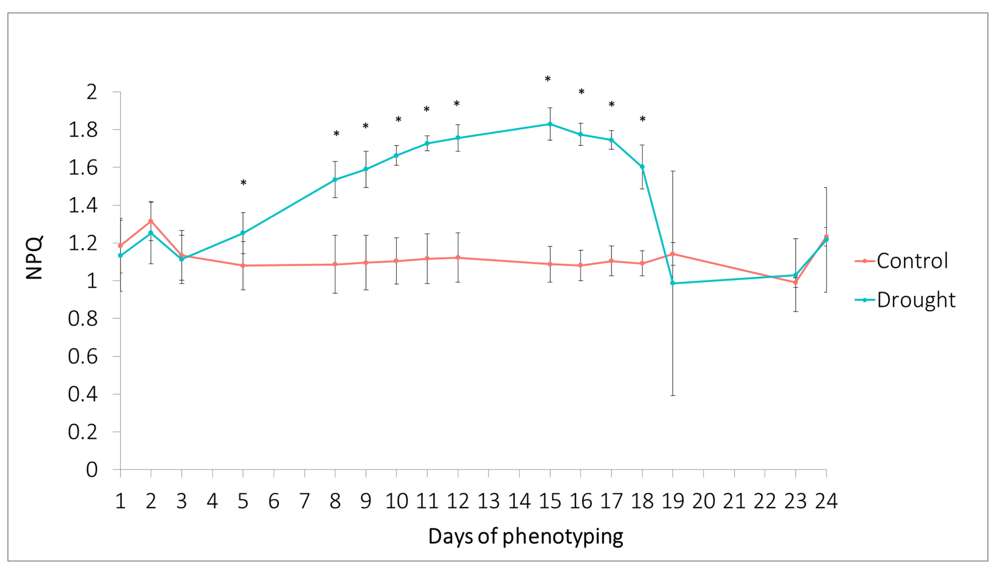


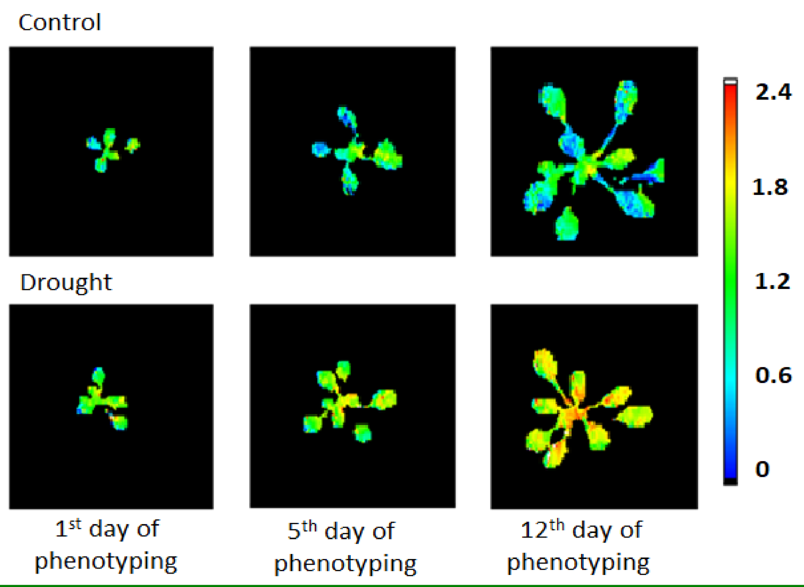
Kinetic chlorophyll fluorescence imaging was used for analysis of photosynthetic performance of the plants in drought and control conditions. Here we show that maximum quantum yield **QY_max (Fv/Fm)** was reduced only in very late stage of the stress when the plants started to wilt and as such Fv/Fm cannot be used as sensitive parametr to monitor photosynthetic performance in the plants.





Fq'/Fm' parametr that quantifies the steady-state PSII quantum yield in light was reduced in the early phase of the drought stress. Statistically significant differences were observed after 10 days from last watering of stressed plants (phenotyping day 8).





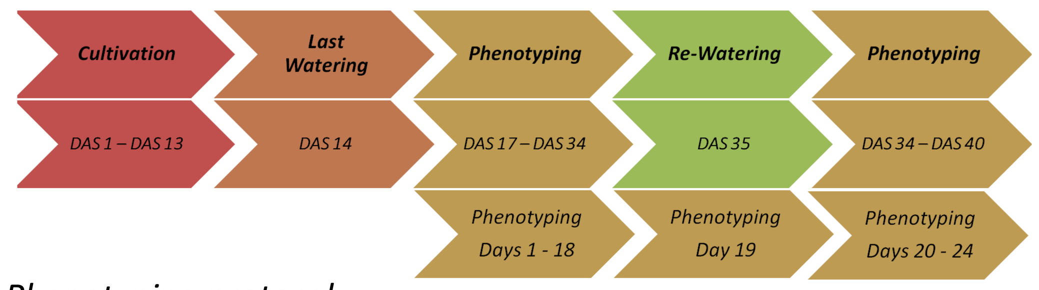
When looking for the fluorescence parameter quickly describing changes induced by drought stress, regulatory light-induced heat dissipation (**NPQ**, non-photochemical quenching) was found out to be the best in our experiment. Statistical significant differences were present 7 days after last watering of stressed plants (phenotyping day 5).

Analysis of variance (ANOVA): *P < 0.05.

Materials and Methods

Plant material, cultivation and handling

Arabidopsis thaliana, accession Columbia seeds were sown- out into the fully soaked soil Klasmann 2 and placed into the fridge (4°C, dark) for 3 days. After stratification, pots were cultivated in FytoScope – Walk In growing chamber (Photon Systems Instruments, Czech Republic) under cool-white LED and far-red LED light of 120 μmol.m^{-2}.s^{-1} light intensity with 14 hours light and 10 hours night light regime. Temperature was set to 22° C/day and 19°C /night, air humidity 60%. Twenty individuals of 7 days old (7 DAS) plants were transplanted to 180 ml pots and 60 g of soil at the stage of 1st true emerging leaf. The soil was watered 3 hours prior the transplantation to 60 percent relative water content (RWC) or so called fiel capacity and this eas kept to DAS 14, when the last watering of treated plants was done. The control plants were still watered to 60%. Plants were then grown under this conditions for 21 days. On DAS 35, where the treated plants were evidently suffering by the drought, the re-watering step was done. The experiment was ended 5 days after re-watering.

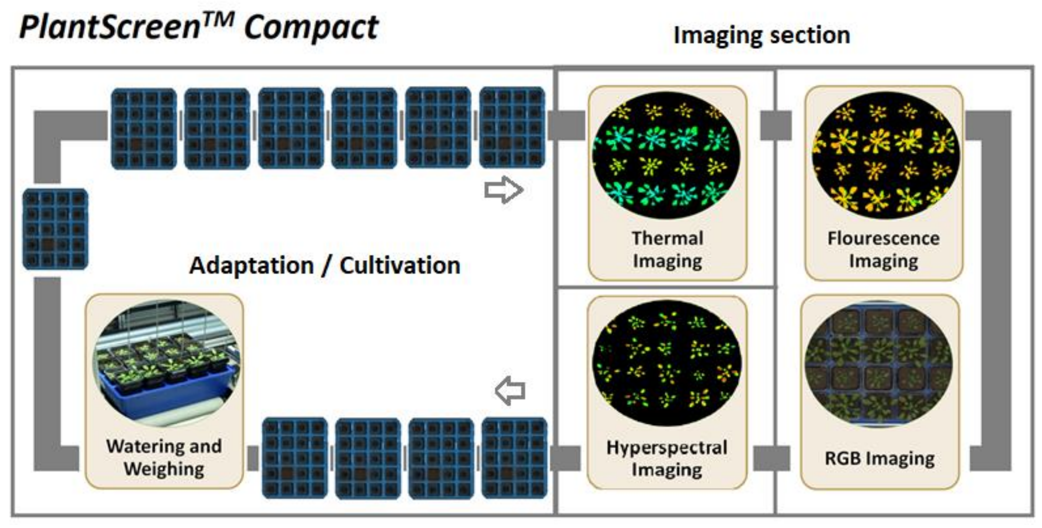


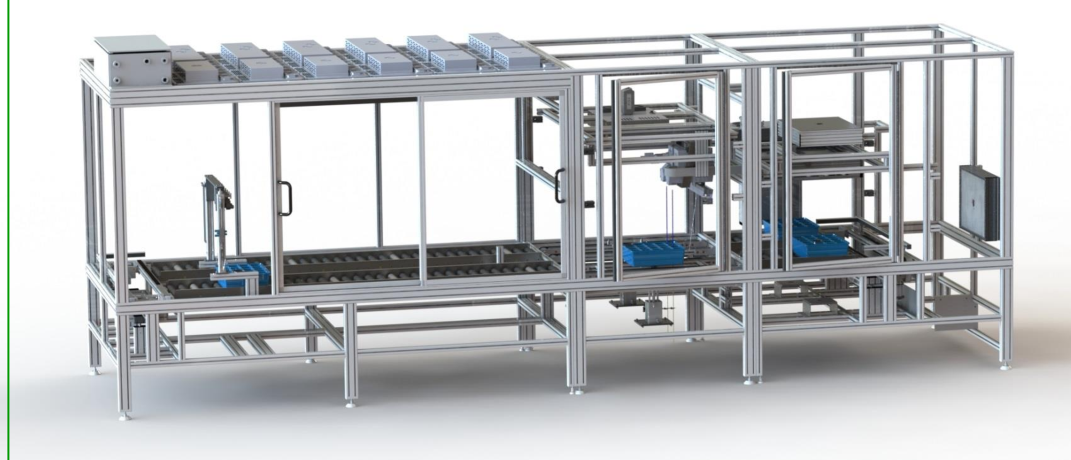
Phenotyping protocol

For the phenotyping, plants were moved from the growing chamber into the PlantScreen^{TM} Compact System. First step in the protocol was the adaptation of plants in the PS System for 30 minutes. Air temperature was set to 22°C and humidity 40%. Following protocol was then started:

1. Thermal imaging- one image in darkness (thermal camera)
2. 15 minutes dark adaptation
3. Fluorescence Imaging – Light curve protocol using different levels of photon irradiances (details e.g. in Awlia et al. 2016)
4. RGB imaging – top view
5. WW (watering and weighing) step as described above (60% for the Control vs. 0% for Stress)

After phenotyping, plants were moved back into the growing chamber.





Data Analysis

PSI PlantScreen Data Analyzer software was used for automated image processing analysing and export of numeric values of the data obtained with the **PlantScreen^{TM} Systems**. This software allows vizulization of the data in graphs, as well as inspecting of the pictures from the imaging sensors. Data were then exported in form of tables and further investigated in 'MVApp' statistical application based on R programming language (Julkowska et al. 2018).

Conclusions

PlantScreen^{TM} Compact System integrates fast and accurate multi-sensoric tools, which are required for efficient and effective plant high-throughput phenotyping. Combination of thermal imaging, RGB imaging and fluorescence camera enables integrative analysis of the stress level affecting the plant. Our data show that rapid after drought stress initiation (4 days after last watering) leaf surface temperature is higher in stressed plants reflecting stomata closure and non-photochemical quenching is increased followed by decline in PSII efficiency in light harvesting.

References

Awlia, M., Nigro, A., Fajkus, J., Schmoeckel, S. M., Negrão, S., Santelia, D., ... & Panzarová, K. (2016). High-throughput non-destructive phenotyping of traits that contribute to salinity tolerance in Arabidopsis thaliana. *Frontiers in Plant Science*, *7*, 1414.

Julkowska, M.M., Saade, S., Gao, G., Morton, M.J.L., Awlia, M., Tester, M.A., MVApp.pre-release_v2.0 mmjulkowska/MVApp: MVApp.pre-release_v2.0, DOI: 10.5281/zenodo.1067974